## SEQUENCE LISTING

5			
J		(i)	APPLICANT: Tsien, Roger Y. Heim, Roger
10		(ii) Construct	TITLE OF INVENTION: Tandem Fluorescent Protein
		(iii)	NUMBER OF SEQUENCES: 25
15	# [] [] [ <u>"</u> "	(iv)	CORRESPONDENCE ADDRESS:  (A) ADDRESSEE: Fish & Richardson P.C.  (B) STREET: 4225 Executive Square #1400  (C) CITY: San Diego  (D) STATE: California  (E) COUNTRY: USA
20	U V		(E) COUNTRY: USA (F) ZIP: 92037
25	sos .c	(v)	COMPUTER READABLE FORM:  (A) MEDIUM TYPE: Floppy disk  (B) COMPUTER: IBM PC compatible  (C) OPERATING SYSTEM: PC-DOS/MS-DOS  (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
30		(vi)	CURRENT APPLICATION DATA: (A) APPLICATION NUMBER: (B) FILING DATE: (C) CLASSIFICATION:
35		(viii)	ATTORNEY/AGENT INFORMATION: (A) NAME: Haile, Lisa A. (B) REGISTRATION NUMBER: 38,347 (C) REFERENCE/DOCKET NUMBER: 07257/030001/UC 96-160-1
40		(ix)	TELECOMMUNICATION INFORMATION:  (A) TELEPHONE: 619-678-5070  (B) TELEFAX: 619-678-5099

(1) GENERAL INFORMATION:

		(2)	INFO	RMAT	ION	FOR :	SEQ	ID N	0:1:								
5			(i)	(A (B (C	UENC ) LE ) TY ) ST	NGTH PE: RAND	: 71 nucl EDNE	7 ba eic SS:	se p acid sing	airs							
.0			(ii)	MOL	ECUL	E TY	PE:	cDNA									
.5			(ix)	(A	TURE ) NA ) LC	ME/K			17								
	H		(xi)	SEÇ	UENC	E DE	SCRI	PTIC	N: S	EQ I	D NO	:1:					
30		ATG	AGT	AAA	GGA	GAA	GAA	CTT	TTC	ACT	GGA	GTT	GTC	CCA	TTA	CTT	GTT
	W I	Met	18 Ser	Lys	Gly	Glu	Glu	Leu	Phe	Thr	Gly	Val	Val	Pro	Ile	Leu	Val
25	UT .	1				5					10					15	
		GAA	TTA	GAT	GGT	GAT	GTT	AAT	GGG	CAC	AAA	TTT	TCT	GTC	AGT	GGA	GAG
30		Glu	96 Leu	Asp	Gly	Asp	Val	Asn	Gly	His	Lys	Phe	Ser	Val	Ser	Gly	Glu
	•				20					25					30		
35		GGT	GAA	GGT		GCA	ACA	TAC	GGA	AAA	CTT	ACC	CTT	AAA	TTT	ATT	TGC
		Gly	144 Glu	Gly	Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Phe	Ile	Cys
40				35					40					45			
45		ACT	ACT	GGA	. AAA	CTA	CCT	GTT	CCA	TGG	CCA	ACA	CTT	GTC	ACT	ACT	TTC
		1 Thr	92 Thr	Gly	Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr	Phe
			50	)				55					60				

		TCT	TAT	GGT	GTT	CAA	TGC	TTT	TCA	AGA	TAC	CCA	GAT	CAT	ATG	AAA	CGG
5				Gly	Val	Gln		Phe	Ser	Arg	Tyr		Asp	His	Met	Lys	
		65					70					75					80
.0				TTT	TTC	AAG	AGT	GCC	ATG	CCC	GAA	GGT	TAT	GTA	CAG	GAA	AGA
			88 Asp	Phe	Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val	Gln	Glu	Arg
l5						85					90					95	
	HO	ACT	ATA	TTT	TTC	AAA	GAT	GAC	GGG	AAC	TAC	AAG	ACA	CGT	GCT	GAA	GTC
50			336 Ile	Phe	Phe	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr	Arg	Ala	Glu	Val
	U O				100					105					110		
25				GAA	GGT	GAT	ACC	CTT	GTT	AAT	AGA	ATC	GAG	TTA	AAA	GGT	ATT
	F N		384 Phe	Glu	Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Lys	Gly	Ile
30				115					120					125			
	3 12	GAT	TTT	AAA	GAA	GAT	GGA	AAC	ATT	CTT	GGA	CAC	AAA	TTG	GAA	TAC	AAC
35			432 Phe	Lys	Glu	Asp	Gly	Asn	Ile	Leu	Gly	His	Lys	Leu	Glu-	Tyr	Asn
			130	<u>ن</u>	•			135	•				140		-		÷.

		TAT	AAC	TCA	CAC	AAT	GTA	TAC	ATC	ATG	GCA	GAC	AAA	CAA	AAG	AAT	GGA
5		Tvr	480 Asn	Ser	His	Asn	Val	Tvr	Ile	Met	Ala	Asp	Lvs	Gln	Lvs	Asn	Glv
J		145					150	-1-				155	-10	<b>J</b>	210		160
					•												
10		ATC	AAA	GTT	AAC	TTC	AAA	ATT	AGA	CAC	AAC	ATT	GAA	GAT	GGA	AGC	GTT
			528 Lys	Val	Asn	Phe	Lys	Ile	Arg	His	Asn	Ile	Glu	Asp	Gly	Ser	Val
15						165					170					175	
	H																
				GCA	GAC	CAT	TAT	CAA	CAA	AAT	ACT	CCA	ATT	GGC	GAT	GGC	CCT
20			576 Leu	Ala	Asp	His	Tyr	Gln	Gln	Asn	Thr	Pro	Ile	Gly	Asp	Gly	Pro
					180					185					190		
25		GTC	ርጥጥ	ጥΤΔ	CCA	GAC	ልልሮ	СУТ	ጥልሮ	ርጥር	ፐርር	aca	CAA	TCT	GCC	СТТ	ፐርር
			24		00	00		0.11		010	100		0.2.	-01	000		100
30	IJ O			Leu	Pro	Asp	Asn	His	Tyr	Leu	Ser	Thr	Gln	Ser	Ala	Leu	Ser
				195					200					205			
		AAA	GAT	CCC	AAC	GAA	AAG	AGA	GAC	CAC	ATG	GTC	CTT	CTT	GAG	TTT	GTA
35			672														
		Lys		Pro	, Asn	Glu	Lys		Asp	His	Met	Val		Leu	Glu	Phe	<u>V</u> al
40			210					215					220				
		ACA	GCT	GCT	GGG	ATT	ACA	CAT	GGC	ATG	GAT	GAA	CTA	TAC	AAA	TA	
45		717 Thr		Ala	Glv	· Ile	Thr	His	Glv	Met	Asp	Glu	Leu	Tyr	Ivs		
		225			1		230		1			235		-1-	_, 5		

5			(	i) S			GTH: E: a	238 minc		no a d		:					
.0			·			ULE		_									
			(χ	ci). S	EQUE	ENCE	DESC	RIPI	CION:	SEÇ	DID	NO:2	! <b>:</b>				
		Met 1	Ser	Lys	Gly	Glu 5	Glu	Leu	Phe	Thr	Gly 10	Val	Val	Pro	Ile	Leu 15	Val
L5	j.	Glu	Leu	Asp	Gly 20	Asp	Val	Asn	Gly	His 25	Lys	Phe	Ser	Val	Ser 30	Gly	Glu
30		Gly	Glu	Gly 35	Asp	Ala	Thr	Tyr	Gly 40	Lys	Leu	Thr	Leu	Lys 45	Phe	Ile	Cys
		Thr	Thr 50	Gly	Lys	Leu	Pro	Val 55	Pro	Trp	Pro	Thr	Leu 60	Val	Thr	Thr	Phe
25		Ser 65	Tyr	Gly	Val	Gln	Cys 70	Phe	Ser	Arg	Tyr	Pro 75	Asp	His	Met	Lys	Arg 80
	H N S	His	Asp	Phe	Phe	Lys 85	Ser	Ala	Met	Pro	Glu 90	Gly	Tyr	Val	Gln	Glu 95	Arg
30		Thr	Ile	Phe	Phe 100	Lys	Asp	Asp	Gly	Asn 105	Tyr	Lys	Thr	Arg	Ala 110	Glu	Val
35		Lys	Phe	Glu 115	Gly	Asp	Thr	Leu	Val 120	Asn	Arg	Ile	Glu	Leu 125	Lys	Gly	Ile
		Asp	Phe 130		_Glu	Asp	Gly	Asn 135	Ile	Leu	Gly	His	Lys 140	Leu	Glu	Tyr	Ąsn
40		Tyr 145	Asn	Ser	His	Asn	Val 150		Ile	Met	Ala	Asp 155	Lys	Gln	Lys	Asn	Gly 160
4.5		Ile	Lys	Val	Asn	Phe 165	Lys	Ile	Arg	His	Asn 170		Glu	Asp	Gly	Ser 175	Val
45		Gln	Leu	Ala	Asp 180	His	Tyr	Gln	Gln	Asn 185		Pro	Ile	Gly	Asp 190	Gly	Pro
50		Val	Leu	Leu 195		Asp	Asn	His	Tyr 200		Ser	Thr	Gln	Ser 205	Ala	Leu	Ser

(2) INFORMATION FOR SEQ ID NO:2:

	Lys	Asp 210	Pro	Asn	Glu	Lys	Arg 215	Asp	His	Met	Val	Leu 220	Leu	Glu	Phe	Val
5	Thr 225	Ala	Ala	Gly	Ile	Thr 230	His	Gly	Met	Asp	Glu 235	Leu	Tyr	Lys		
10	(2)	INFO	SEQ ()	QUENC A) LI 3) T	FOR CE CI ENGTI YPE: TRANI	HARA( H: 8 amin	CTER amin	ISTIC no ac	CS: cids							
15					OPOLO				916							
20					LE TY				SEQ :	ID NO	0:3:					
25		Ser 1	Glı	n Ası	а Ту	r Pro 5	o Ile	e Vai	l Gl	Y						
	(2)	INFO	ORMA!	rion	FOR	SEQ	ID 1	NO:4	:							
30		(i)	() ()	A) L1 B) T1 C) S1	CE CI ENGTI YPE: IRANI OPOLA	H: 10 amin DEDN	0 am. no a ESS:	ino d cid sin	acid	S						
35		(ii)	MO	LECU	LE T	YPE:	pep	tide								

			(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
5			Lys Ala Arg Val Leu Ala Glu Ala Met Ser 1 5 10
		(2)	INFORMATION FOR SEQ ID NO:5:
10			<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 10 amino acids</li> <li>(B) TYPE: amino acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>
15			(ii) MOLECULE TYPE: peptide
20			(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:  Pro Ser Pro Arg Glu Gly Lys Arg Ser Tyr 1 5 10
25		(2)	INFORMATION FOR SEQ ID NO:6:
30	. Ozzs		<ul><li>(i) SEQUENCE CHARACTERISTICS:</li><li>(A) LENGTH: 5 amino acids</li><li>(B) TYPE: amino acid</li><li>(C) STRANDEDNESS: single</li><li>(D) TOPOLOGY: linear</li></ul>
	T.		(ii) MOLECULE TYPE: peptide
35			(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
			Tyr Val Ala Asp Gly

		(2)	INFORMATION FOR SEQ ID NO:7:
5			<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 8 amino acids</li> <li>(B) TYPE: amino acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>
10			(ii) MOLECULE TYPE: peptide
			(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7
15	¥		Met Phe Gly Gly Ala Lys Lys Arg 1 5
20		(2)	INFORMATION FOR SEQ ID NO:8:
25	o U		<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 10 amino acids</li> <li>(B) TYPE: amino acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>
			(ii) MOLECULE TYPE: peptide
30			(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8
35			Gly Val Val Asn Ala Ser Ser Arg Leu A 1 5 1
		(2)	INFORMATION FOR SEQ ID NO:9:
40			<ul><li>(i) SEQUENCE CHARACTERISTICS:</li><li>(A) LENGTH: 9 amino acids</li><li>(B) TYPE: amino acid</li><li>(C) STRANDEDNESS: single</li><li>(D) TOPOLOGY: linear</li></ul>
45			(ii) MOLECULE TYPE: peptide
			(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9
50			Leu Ile Ala Tyr Leu Lys Lys Ala Thr

_		(2)	INFOR	MATION FOR SEQ ID NO:10:	
.0			(i)	SEQUENCE CHARACTERISTICS:  (A) LENGTH: 7 amino acids  (B) TYPE: amino acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
			(ii)	MOLECULE TYPE: peptide	
L5			(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:10:	
20			Val 1	Lys Met Asp Ala Glu Phe 5	
		(2)	INFOR	RMATION FOR SEQ ID NO:11:	
25			(i)	SEQUENCE CHARACTERISTICS:  (A) LENGTH: 17 amino acids  (B) TYPE: amino acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
30			(ii)	MOLECULE TYPE: peptide	
35			(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:11:	
33		Arg	Phe	Leu Ala Glu Gly Gly Gly Val Arg Gly Pro Arg Val	Val Gl
		мц	1	5 10	15
40	•		His		

5		·	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 13 amino acids</li> <li>(B) TYPE: amino acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>
.0			(ii) MOLECULE TYPE: peptide
			(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
.5	H		Asp Arg Val Tyr Ile His Pro Phe His Leu Val Ile His 1 5 10
		(2)	INFORMATION FOR SEQ ID NO:13:
20			<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 8 amino acids</li> <li>(B) TYPE: amino acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>
			(ii) MOLECULE TYPE: peptide
30			(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
35			Lys Pro Ala Leu Phe Phe Arg Leu 1 5
		(2)	INFORMATION FOR SEQ ID NO:14:
40			<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 30 amino acids</li> <li>(B) TYPE: amino acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>

(ii) MOLECULE TYPE: peptide

45

(2) INFORMATION FOR SEQ ID NO:12:

			(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
		Com	Gln Pro Leu Gly Gln Thr Ser Leu Met Lys Arg Pro Pro Gly Phe
5		Ser	1 5 10 15
			Pro Phe Arg Ser Val Gln Val Met Lys Thr Gln Glu Gly Ser 20 25 30
.0		(2)	INFORMATION FOR SEQ ID NO:15:
.5	¥		<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 5 amino acids</li> <li>(B) TYPE: amino acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>
30			(ii) MOLECULE TYPE: peptide
			(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
25			Gly Gly Gly Ser 1 5
30	U)	(2)	INFORMATION FOR SEQ ID NO:16:
35	thung.		<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 22 amino acids</li> <li>(B) TYPE: amino acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>
			(ii) MOLECULE TYPE: peptide
40			(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
			Gly Gly Gly Gly Gly Ser Met Phe Gly Gly Ala Lys Lys Arg
45		Ser	1 5 10 15
			Gly Gly Gly Gly Gly 20
50		(2)	INFORMATION FOR SEQ ID NO:17:

5			(1)		STR	IGTH: PE: & RANDE	: 35 amino EDNES		no ad id singl	cids							
			(ii)	MOLE	CULE	E TYP	PE: 1	pepti	ide								
.0			(xi)	SEOU	JENCE	E DES	SCRII	PTION	N: SI	EO II	O NO	:17:					
					Arg								Val	Glu	Glu	Leu	Leu
_		Ser		OIII	n.g	MCC		OIII	БСС	Giu	лор		vai	Giu	Gru	neu	
.5			1				5					10					15
	j-i	Val	Lys	Asn	Tyr	His	Leu	Glu	Asn	Glu	Val	Ala	Arg	Leu	Lys	Lys	Leu
		val				20					25					30	
20			Gly	Glu	Arg 35												
25		(2)	INFO	RMAT:	ION E	FOR S	SEQ :	ID NO	0:18	:							
30			(i)	(A)	UENCE ) LEM ) TYM ) STM ) TOM	NGTH PE: 6 RANDI	: 6 amin	amin	o ac: id sing:	ids							
35			(ii)	MOL	ECULI	E TY	PE:	pept:	ide								
			(xi)	SEQ	UENCI	E DE	SCRI	PTIO	N: S	EQ I	D NO	:18:					~'
40			Ser 1	Lys	Val	Ile	Leu 5	Phe						,			

		(2)	INFORMATION FOR SEQ ID NO:19:										
5			<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 22 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>										
.0			(ii) MOLECULE TYPE: DNA (oligonucleotide)										
			(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:										
.5	¥	GGATCCCCCC GCTGAATTCA TG 22											
o U		(2)	INFORMATION FOR SEQ ID NO:20:										
25	rooszas "o		<ul><li>(i) SEQUENCE CHARACTERISTICS:</li><li>(A) LENGTH: 15 base pairs</li><li>(B) TYPE: nucleic acid</li><li>(C) STRANDEDNESS: single</li><li>(D) TOPOLOGY: linear</li></ul>										
20			(ii) MOLECULE TYPE: DNA (oligonucleotide)										
30			(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:										
35		AAA	TAATAAG GATCC 15										
		(2)	INFORMATION FOR SEQ ID NO:21:										
40			<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 33 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>										

(ii) MOLECULE TYPE: DNA (primer)

			(x1) SEQUENCE DESCRIPTION: SEQ ID NO:21:
_		GGTA	AAGCTTT TATTTGTATA GTTCATCCAT GCC 33
5		(2)	INFORMATION FOR SEQ ID NO:22:
LO			<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 24 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>
L <b>5</b>			(ii) MOLECULE TYPE: DNA (primer)
			(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
30	-	AGA!	AAGGCTA GCAAAGGAGA AGAA 24
		(2)	INFORMATION FOR SEQ ID NO:23:
25			<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 25 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>
30	VO.		(ii) MOLECULE TYPE: DNA (primer)
35			(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23
		TCA	GTCTAGA TTTGTATAGT TCATC 25
40		(2)	INFORMATION FOR SEQ ID NO:24:
45			<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 10 amino acids</li> <li>(B) TYPE: amino acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>
			(ii) MOLECULE TYPE: peptide

			(X1)	SEQU	JENCE	E DES	CRIE	PITO	v: Si	EQ II	) NO:	24:						
5			Met 1	Arg	Gly	Ser	His 5	His	His	His	His	His 10						
		(2)	INFORMATION FOR SEQ ID NO:25:															
10		<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 26 amino acids</li> <li>(B) TYPE: amino acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>																
15			(ii)	MOLE	ECULE	E TYI	PE: p	pept:	ide									
20			(xi)	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:														
20	<b>1</b>	Asp	Ser	Ser	Met	Thr	Gly	Gly	Gln	Gln	Met	Gly	Arg	Asp	Leu	Tyr	Asp	
		nop	1				5					10					15	
25			Asp	Asp	Lys	Asp 20	Pro	Pro	Ala	Glu 25→	Phe							
30		SEQ.	12	No.	: 26 Pro	Leu	Tyr	Lys	Asp	Ala	Thr	Asp	Phe				·	
35		SEQ.	14	No.	: 27 Asn	Pro	Leu	Tyr	Lys	Asp	Ala	Thr	Ser	Asp	Phe	-		
40		SEQ.	ID. 16 Gly		: 28 Ala	Asn	Pro	Leu	Tyr	Lys	Asp	Ala	Thr	Ser	Gly	Asp	Phe	
45		SEQ.			: 29 Ala	Asn	Pro	Leu	Tyr	Lys	Asp	Ala	Thr	Ser	Gly	Ser	Thr	

LO

SEQ. ID. No.: 30
20
Gly Thr Ala Asn Pro Leu Tyr Lys Asp Ala Thr Ser Gly Ser Thr
Gly Ser Asp Phe

SEQ. ID. No.: 31
22
Gly Thr Ala Asn Pro Leu Tyr Lys Asp Ala Thr Ser Gly Ser Thr
Gly Ser Gly Ser Asp Phe